PREVALENCE OF MALIGNANCIES IN INFLAMMATORY BOWEL DISEASE PATIENTS ON IMMUNOSUPPRESSANTS

A research report submitted to the Faculty of Health Sciences, Wits University, Johannesburg, in partial fulfillment of the requirements for the degree of Masters of Medicine (MMED) in the department of Internal Medicine.

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JOHANNESBURG, 2019

DECLARATION

I, Loyiso Sobazile, student number 1586450, hereby declare that this Research Report is my own, independent work. It is being submitted for the Masters of Medicine (MMED) degree at Wits University, Johannesburg. It has not been submitted before for any degree or examination at any other institution for higher learning.

Signature: _____

Date: 19 December 2019

DEDICATION

I would like to dedicate this to my loving mother, Thozama Sobazile and my caring grandmother, Nokuzola Millicent Sobazile.

ABSTRACT

Crohn's disease (CD) and ulcerative colitis (UC) are chronic idiopathic inflammatory bowel diseases (IBD) with distinct and overlapping clinical and pathological features. Most CD patients and some UC patients will require immunosuppressive (IS) therapy to control bowel inflammation and the resulting complications. Unfortunately IS therapy has been shown, in IBD and in other settings such as solid organ transplant, to increase the risk of extra-intestinal malignancies. To understand the association between cancer and IS therapy in IBD patients, I have conducted a retrospective single-center study in an urban area in South Africa.

STUDY DESIGN AND METHODS: This retrospective record review study was done at Charlotte Maxeke Johannesburg Academic Hospital Gastroenterology Clinic (CMJAH) affiliated with Wits University. A total of 202 clinic files from our IBD cohort database were retrieved and analyzed. The diagnosis of IBD was made between 1980 and January 2017. Commonly used drugs were azathioprine (AZA) and methotrexate (MTX). Information about the histopathological diagnosis of IBD and malignancy was confirmed using data from National Health Laboratory Services (NHLS) and Disa*Lab.

RESULTS: Among the 99 patients who received IS therapy, 8 (8.08%) patients developed cancer. Age at IBD diagnosis (adjusted hazard ratio [HR] 1.05, 95% CI 1.00 – 1.10) and exposure to IS therapy (HR 11.01, 95% CI 1.27 – 95.28) had significant

associations with cancer development (both $p<0.05$). Six of the patients who developed cancer were on thiopurines. Non-melanoma skin cancer (NMSC) and renal cell carcinoma (RCC) were more prevalent.

CONCLUSION: Patients on IS therapy are 11 times more likely to develop malignancies than those who are not. Patients with IBD on IS should be counselled about the risk of cancer. Regular age-appropriate cancer screening is recommended.

ACKNOWLEDGEMENTS

I would like to extend my gratitude to:

My supervisors Dr Manoko Elizabeth Seabi and Wisdom Forward Mudombi

National Health Laboratory Service and Disa*Lab

Dr Mazvita Sengayi for her invaluable input

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ABREVIATIONS

6-MP - 6-Mecarptopurine

AML - Acute myeloid leukemia

AZA - Azathioprine

BCC - Basal cell carcinoma

CD – Crohn’s disease

CMJAH - Charlotte Maxeke Johannesburg Academic Hospital

CRC - Colorectal cancer

CSA - Cyclosporine

EBV – Epstein-Barr Virus

ECCO - European Crohn’s and Colitis Organization

FK506 - Tacrolimus

IBD - Inflammatory bowel disease

IARC - Agency for Research on Cancer

IS - Immunosuppressive

LPD - Lymphoproliferative disorders

MDS - Myelodysplastic syndromes

MSC - Melanoma skin cancer

MTX - Methotrexate

NMSC - Non-melanoma skin cancer

NHL – Non-Hodgkin’s lymphoma

NHLS - National Health Laboratory Services

RCC - Renal cell carcinoma

SCC - Squamous cell carcinoma

TNF - Tumor necrosis factor

UC - Ulcerative colitis

CHAPTER 1

1. INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) are idiopathic chronic inflammatory gastrointestinal diseases with varying episodes of active inflammation and disease quiescence. They affect approximately 0.4% of the North American and West European population (1). Data regarding incidence and prevalence of IBD in developing nations such as South Africa is inadequate. With the increase in industrialization and adoption of a Western lifestyle it is expected to be on the rise (2-4) .Immunosuppressive (IS) therapy is widely used to induce and maintain clinical and endoscopic/pathological disease remission (5). Given the prolonged and sometimes lifelong courses of such therapies and the ageing IBD population, the risk of carcinogenesis is increased (6-8).

Azathioprine (AZA) and 6-mecaptopurine (6-MP) are the thiopurines most commonly used to treat steroid-resistant UC and CD (5, 8). Azathioprine has been classified as a carcinogen by the International Agency for Research on Cancer (9). In most cases where the disease is refractory to thiopurines or cannot be used due to adverse side effects, Methotrexate (MTX) and the calcineurin inhibitors (Cyclosporin A [CSA], Tacrolimus [FK506]) have shown potential (10-12) .The risk of neoplasia with thiopurines is well described in inflammatory conditions such as rheumatoid arthritis (13)

and in transplant medicine (14, 15). However, in IBD patients on IS therapy, data is conflicting. In addition to this, most research in current literature has been done in American and European cohorts (6-8). There is little knowledge about the risk of cancer in IBD patients on IS therapy in the African continent.

1.1 Hematological malignancies

Immunosuppressive (IS) therapies such as azathioprine, after long-term exposure, exert their carcinogenic effects by altering cellular DNA, activating oncogenes, interfering with control of infections caused by mutagenic viruses and by reducing the surveillance of tumor cells (8, 16-19). The duration and degree of immunosuppression among other host factors such as age, gender and prior malignancies, are important risk factors for the development of new or recurrent malignancies (8, 20).

The risk of developing non-Hodgkin's lymphoma (NHL) solely from IBD is negligible (21), except for a primary intestinal lymphoma strongly associated with the increased duration of bowel inflammation in CD (21, 22). Breaches in immune surveillance of Epstein-Barr virus (EBV) due to thiopurine exposure increases risk of EBV-associated lymphomas (17, 18, 23). Defective cytotoxic T-cell function in immunocompromised hosts leads to unrestricted lymphoproliferation of infected B cells (18). High EBV viral load is associated with increased risk of lymphomagenesis in HIV and post organ

transplant (15, 24, 25). AZA has been associated with an increased risk of DNA mismatch repair defects in myeloid cells, thus promoting clonal expansion. This has a potential to evolve into acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) as demonstrated by Offman and colleagues in transplant recipients (26).

A multi-national prospective study showed a 5-fold incident risk of developing lymphomas in thiopurine exposed cohorts (CESAME, 2009) (23). Consistent with the CESAME study, a large meta-analysis of 6 studies in 2005, revealed a 4-fold higher risk of LPD in IBD patients treated with thiopurines (27). A 2013 nationwide retrospective cohort study showed the risk of LPD in thiopurine-experienced cases was 4-fold higher in comparison to patients who have not been exposed. They also found that the risk increases with successive years of therapy and that discontinuing therapy reduces risk (28). A prospective study of 782 patients with IBD between 1990 and 1999, concluded that the mean duration of IBD at the time of NHL diagnosis was 3.1 years, and the mean duration between initiation of IS and diagnosis of NHL was 20 months (29). Out of 238 patients who received IS four developed NHL; a risk almost 6-fold compared to the general population. Cyclosporine (CSA) was one of the immunosuppressants the cohort was exposed to. A 2015 meta-analysis revealed that the risk of lymphoma was greatest after a year of exposure. They also concluded that patients over the age of 50 and men younger than 35 were high risk groups (30).

As stated above, in a study by Offman, thiopurines have been proven to increase risk of leukemogenesis in the post-transplant setting (26). A large 2014 prospective

observational study in France showed a 7-fold increased risk of myeloid disorders in past users of thiopurines (31). They followed up a cohort of 19,486 patients for a period of 3 years. Five malignancies were diagnosed: 3 with AML and 2 with MDS.

Cases of fatal post-mononucleosis LPD have been reported in young EBV seronegative males exposed to thiopurines (23, 32). In male IBD patients younger than 35 years, exposure to thiopurines for a median period of 6 years increases chances of having hepatosplenic T-cell lymphoma. This risk is even higher when thiopurines are combined with anti-TNF (tumor-necrosis factor) agents for at least two years (33, 34).

Methotrexate (MTX) increases risk of lymphoproliferative disorders in rheumatoid arthritis (RA) patients who are EBV seropositive (35, 36). Fortunately these have been shown to spontaneously regress after drug withdrawal (37). However, in IBD, data linking MTX use to lymphomagenesis is extremely limited. In the study cited above done by Farrell, only two patients who developed NHL were exposed to MTX (29).

1.2 Skin cancer

Multiple studies have consistently proven a strong association between thiopurine use and non-melanoma skin cancer (NMSC). Thiopurines such as AZA increase ultraviolet A/B ionizing radiation burden on skin epithelial cells resulting in DNA damage by

oxidative stress (38). Mutations in *PTCH*, a tumor suppressor gene, are linked to the development of basal cell carcinoma (BCC) in sun non-exposed areas of AZA users (38).

A nationwide retrospective cohort study found a 2-fold increase in risk of NMSC in UC patients on thiopurines. The risk significantly increased with number of exposure-years and stopping treatment reduced the risk to baseline (39). A nested case-control study in 2010 concluded that in a United States IBD cohort (53,377 patients) thiopurines increased the risk of NMSC >4-fold compared with IBD patients not on IS therapy (OR=3.56, 95% CI, 2,81-4.50). Even recent (< 3 months) users of thiopurines were at increased risk (40). In a French prospective cohort study of over 19 000 patients with IBD, the risk of NMSC was significantly increased in both current and past users of thiopurines (HR=5.9, 95% CI, 2.1-16.6 and HR=4.1, 95% CI, 1.3-13.3, respectively). Patients not exposed to IS therapy, regardless of their IBD subtype, had the same risk of developing skin cancer as the general population (41). A review article published in NEJM in 2003, showed that recipients of solid organ transplants had a 65- to 250-fold risk of NMSC, SCC in particular (42). The risk was greatly increased in light skinned individuals on high doses of IS therapy such as AZA, Mycophenolate mofetil and lower in those receiving low dose CSA. A 2016 retrospective cohort study in Philadelphia of over 9000 participants with IBD and rheumatoid arthritis (RA) found that the use of MTX increases risk of a second NMSC. One year or more of MTX use increases the risk of a

second NMSC (43). One study reported no increased risk with exposure to MTX although the analysis was limited by a small sample size of 350 patients (44).

The risk of melanoma skin cancer (MSC) per se does not appear to be increased with the use of thiopurines (45, 46). In the study cited above by Kotlyar, there was no significant difference in the incidence of MSC in patients exposed to thiopurines compared to the group not exposed (2/238 vs 5/544; p=0.9) (30). Exposure to anti-TNF biologics has not been proven to increase risk of NMSC in IBD patients (45). However, the risk of melanoma in IBD patients exposed to TNF antagonists is increased (47).

1.3 Other cancers

1.3.1 Urinary tract

The majority of urinary tract cancers include bladder and kidney cancer. In transplant literature the risk of urinary tract cancers is increased in those exposed to immunosuppression (48, 49).

In a large Danish IBD cohort of 45,986 participants, 11% (5,197) were exposed to AZA. The overall risk of cancer in users of AZA was increased. In subgroup analysis, the risk of urinary tract cancers was also increased (rate ratio=2.84,95% CI:1.24, 6.51),

although direct causality cannot be established (50). The CESAME group, in a prospective observational cohort study of over 19,000 IBD patients receiving thiopurines, found a significant risk of urinary tract cancers (p=0.006). There were 10 and 6 cases of kidney and bladder cancer, respectively. Older age, male gender, active smoking and duration of exposure to AZA were associated with increased risk (51).

Renal transplant recipients on maintenance immunosuppression are at increased risk of prostate cancer (52). AZA and calcineurin inhibitors increase risk of locally advanced disease. It is unclear whether the case is the same in IBD patients on immunosuppression.

1.3.2 Cervix

HIV infected women have a substantially higher risk of invasive cervical cancer (53). It accounts for 3% of cancers in female recipients of solid organ transplants (54). Failure of immune surveillance of chronic Human papillomavirus infection in immunocompromised patients increases risk of cervical dysplasia or invasive cancer (55). It remains controversial whether IBD on its own or treated with IS therapy is associated with neoplastic cervical lesions.

In a recent meta-analysis Allegretti and colleagues showed an adequate body of evidence to suggest a high risk of cervical cancer in IBD cases exposed to IS therapy in

comparison to the population at large (56). However, two separate studies failed to show increased risk with exposure to IS therapy (57, 58).

CHAPTER 2

2.1 Study methods and design

A single-center retrospective record review of patient clinic files with a biopsy proven diagnosis of IBD from 1980 to January 2017 was designed. This study was carried out at a quaternary state center, Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), affiliated with Wits University. The Gastroenterology clinic, mostly a referral center, has an inflammatory bowel disease (IBD) cohort of approximately 250 patients in the electronic database. The filing system is arranged in alphabetic order for the convenience of data search. Every file that could be found was manually analyzed, looking specifically at patient demographics, IBD diagnosis, use of immunosuppressive (IS) therapy and whether diagnosis of cancer was documented (**Appendix 2,** page 38). Both the histopathological diagnosis of IBD and cancer were confirmed through National Health Laboratory Services (NHLS) and Disa*Lab via reference barcodes in each file.

The primary objective was to determine the prevalence of malignancies in IBD patients on immunosuppressive therapy. The association between cancer development, age of IBD diagnosis, IBD subtype, duration of exposure to IS therapy, gender and race was also explored. The IS drugs that our group were exposed to were azathioprine (AZA), methotrexate (MTX), cyclosporine (CSA) and tacrolimus (FK506). None of the patients

were on biologic therapy due to lack of availability in this state hospital. Patients who were not on IS therapy were also included in this study and prevalence of malignancies in this patient group was also ascertained.

A data collection sheet was created to extract all relevant information from each file (**Appendix 2,** page 38). Once data collection was completed it was then transferred to Microsoft Excel 2016 spreadsheet software for statistical analysis.

Inclusion Criteria

I. Age 18 years or older
II. Histologically confirmed diagnosis of IBD (UC or CD)
III. IBD diagnosis made between the year 1980 and January 2017

Exclusion Criteria

I. HIV seropositive patients
II. Patients with pre-existing malignancies prior to treatment with immunosuppressive therapy

III. Patients who are diagnosed with a malignancy within 8 weeks of initiation of immunosuppressive therapy

IV. Patients with IBD unclassified (IBDU)

2.2 Description of statistical methods

The prevalence of cancer among IBD patients and other patient characteristics were described using proportions. The distribution of age at diagnosis and duration of treatment was described using a histogram, the range, mean and standard deviation. The spectrum of cancers was described using a pie chart (**Figure 2,** page 24).

Fischer's exact tests were used to explore associations between patient characteristics and the development of cancer. To identify risk factors for developing cancer among IBD patients, univariate and multivariate logistic regression models were used. Univariate models were fitted for age at diagnosis, race, IBD subtype, exposure to immunosuppressants and duration on IS therapy. The multivariate model was adjusted for age at diagnosis, IBD subtype and exposure to IS therapy.

Significance was set at the 5% significance level ($p<0.05$).

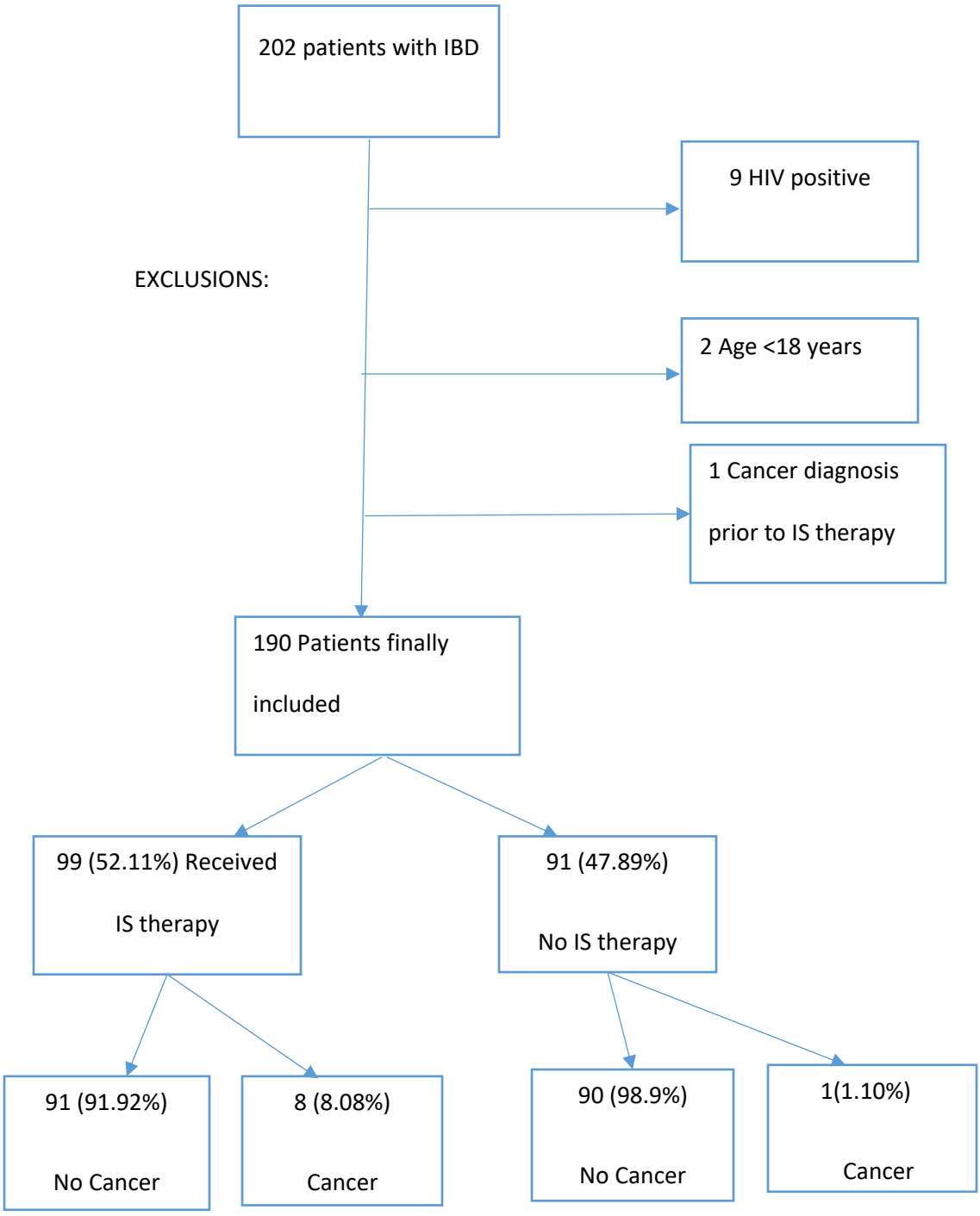
An extensive online search on PubMed was done with the terms “prevalence of malignancies in IBD patients on immunosuppression”, “inflammatory bowel disease and cancer”, “immunosuppression and cancer risk in inflammatory bowel disease”, “thiopurines and cancer risk”, “azathioprine and cancer in IBD”, “6-mercaptopurine and cancer risk in IBD”, “methotrexate and risk of cancer”, “ulcerative colitis”, “Crohn’s disease”, “lymphoma and IBD immunosuppression”, “skin cancer and IBD immunosuppression”, “cervical cancer and IBD treatment”, in order to review current literature on malignancies related to IBD therapy.

2.3 Ethics clearance

Ethical clearance for the study was obtained from Wits University’s Human Research Ethics Committee and is attached in **Appendix 3** (page 40).

CHAPTER 3: Results

3.1 Patient flow chart



3.2 Characteristics of the study population

Out of the 202 IBD cases in our cohort 12 were excluded. Nine were found to be human immunodeficiency virus (HIV) positive, 2 were minors below 18 years and 1 had a malignancy diagnosed before immunosuppressive (IS) therapy. A total of 190 cases were finally included. In all cases, the diagnosis of IBD was made between 1980 and January 2017. Ninety-nine received IS therapy (52.11%) whilst 91 did not (47.89%). A large majority of patients were exposed to azathioprine (n=79, 79.8%) (**Table 1,** page 15). The duration of exposure was more than 2 years in most patients (79.8%). Ulcerative colitis was the most common IBD subtype (n=137, 72.11%) compared to Crohn's disease (n=53, 27.89%).

All patients were followed up at the Gastroenterology clinic at CMJAH. The cohort had more females (n=108) than males (n=82). Seventy-four patients were black, 66 were white and other (50) were either Asian or of mixed ethnicity. The mean age at diagnosis was 50.56 years (Standard deviation ±17.64) (**Figure 1**).

Table 1: Overall Patient Characteristics (N=190)

	Number	%
Demographics		
Age at diagnosis		
<40	98	51.58
40-60	60	31.58
>60	32	16.84
Age at initiation of treatment (N=99)		
<40	52	52.53
40-60	33	33.33
>60	14	14.14
Gender		
Female	108	56.84
Male	82	43.16
Race		
White	66	34.74
Black	74	38.95
Other	50	26.32
Clinical Features		
IBD subtype		
Ulcerative colitis	137	72.11
Crohn's disease	53	27.89
Exposure to immunosuppressants (N=99)		
Thiopurines (Azathioprine)	79	79.8
Methotrexate	14	14.4
Calcineurin inhibitors (Tacrolimus, Cyclosporine)	6	6.06
Duration of exposure (N=105)		
6-12 months	12	12.12
12-24 months	8	8.08
>24 months	79	79.80

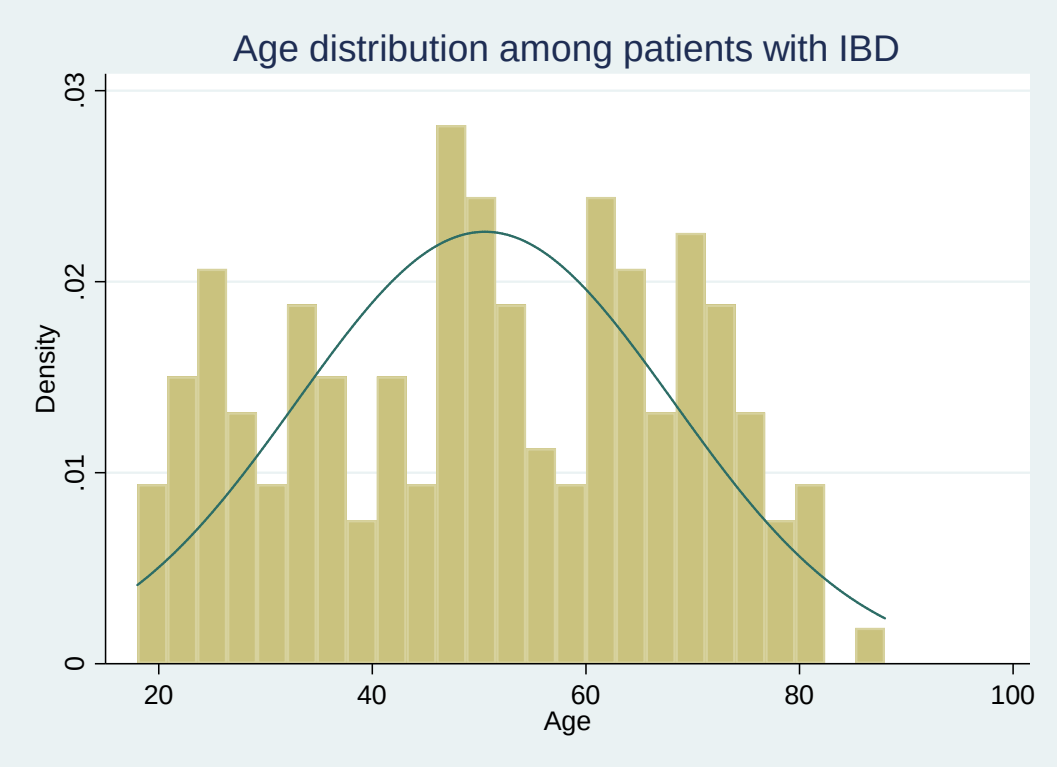


Figure 1: <u>Age distribution among IBD patients</u>

Age followed a normal distribution, range (18-88 years) with a peak in the 50s. Mean age at diagnosis was 50.56 years (Standard deviation ±17.64)

3.3 Spectrum of cancer

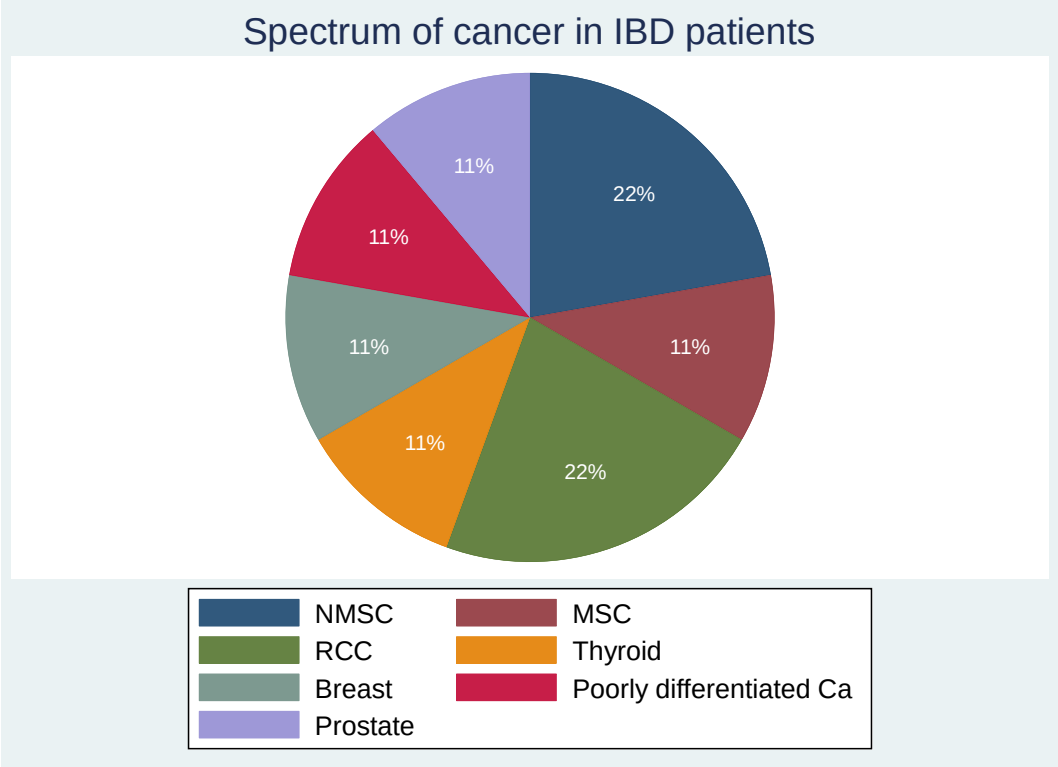


Figure 2: Spectrum of cancer among IBD Patients

A total of 9 (4.74%) out of the 190 IBD patients developed cancer, giving an overall cancer prevalence of 4.74%. NMSC and RCC were the most common cancers.

Among the 99 patients who received immunosuppressive therapy, 8 (8.08%) patients developed cancer (**Table 2,** page 18).

Type of cancer	Number	%
NMSC	2	22.22
MSC	1	11.11
RCC	2	22.22
Thyroid	1	11.11
Breast	1	11.11
Poorly differentiated Ca	1	11.11
Prostate	1	11.11

Table 2: Associations between IBD patient characteristics and development of cancer

	No Cancer		Cancer		P Value
	Number	%	Number	%	
Demographics					
Age at diagnosis					
<40	96	97.96	2	2.04	
40-60	54	90.00	6	10.00	0.080
>60	31	96.88	1	3.13	
Age at initiation of treatment (N=99)					
<40	51	98.08	1	1.92	
40-60	27	81.82	6	18.18	**0.030**
>60	13	92.86	1	7.14	
Gender					
Female	104	96.30	4	3.70	
Male	77	93.90	5	6.10	0.503
Race					
White	60	90.91	6	9.09	
Black	72	97.30	2	2.70	0.159
Other	49	98.00	1	2.00	
Clinical Features					
IBD phenotype					
Ulcerative colitis	131	95.62	6	4.38	
Crohn's disease	50	94.34	3	5.66	0.711
Exposure to immunosupressants					
No	90	98.90	1	1.10	
Yes	91	91.92	8	8.08	**0.036**
Duration of exposure (N=105)					
6-12 months	12	100	0	0	
12-24 months	8	100	0	0	0.641
>24 months	71	89.87	8	10.13	

Age at initiation of treatment and exposure to immunosuppressants had significant associations with cancer development.

Table 3: Risk factors for developing cancer in IBD patients

	Univariate Logistic Regression Model		Multivariate Logistic Regression Model	
	Odds Ratio (95% CI)	P value	Odds Ratio (95%CI)	P value
Age at diagnosis (years)	1.04 (0.99 – 1.09)	0.058	1.05 (1.00 – 1.10)	**0.029**
Race				
Black	Reference		-	
White	3.60 (0.70 – 18.49)	0.125		
Other	0.73 (0.06 – 8.32)	0.803		
IBD subtype				
Ulcerative colitis	Reference		Reference	
Crohn's disease	1.31 (0.31 – 5.44)	0.710	0.78 (0.17 – 3.51)	0.751
Exposure to immunosuppressants				
No	Reference		Reference	
Yes	7.91 (0.97 – 64.56)	0.053	11.01 (1.27 – 95.28)	**0.029**
Duration of exposure (N=105)	1.03 (0.95 – 1.12)	0.417	-	

In univariate logistic regression, age at diagnosis, race, IBD subtype and exposure to immunosuppressants were not associated with developing cancer. In multivariate logistic regression we adjusted for age at diagnosis, IBD type and exposure to

immunosuppressants. Increasing age at diagnosis and exposure to immunosuppressants were independent risk factors for cancer development. Patients who received immunosuppressants were 11 times more likely to develop cancer than those who did not.

Table 4: <u>Cancer prevalence by IBD type and drug exposure</u>

	Ulcerative Colitis (N=137)		Crohn's Disease (N=53)	
Cancer type	Drug exposure (N=56) n (%)	No drug exposure (N=81) n (%)	Drug exposure (N=43) n (%)	No drug exposure (N=10) n (%)
NMSC	1 (1.23%)	0	1 (2.32%)	0
MSC	1 (1.23%)	0	0	0
RCC	2 (2.47%)	0	0	0
Thyroid	1 (1.23%)	0	0	0
Prostate	1 (1.23%)	0	0	0
Breast	0	0	1 (2.32%)	0
Poorly differentiated	0	0	0	1 (10.0%)
Totals – all cancers	6 (7.41%)	0	2 (4.65%)	1 (10.0%)

Table 5: Characteristics of prevalent cancer cases

Number	Cancer	Age (years)	Race	Gender	IBD phenotype	Age at diagnosis	Immunosuppressive therapy
Not exposed to immunosuppressive therapy							
1.	Follicular lymphoma	63	Indian	Male	UC	53	NA
Exposed to immunosuppressive therapy							
2.	RCC	65	Black	Female	UC	50	AZA
3.	Thyroid cancer	69	Black	Male	UC	55	AZA
4.	NMSC	71	White	Female	CD	60	AZA
5.	RCC	48	White	Male	UC	44	AZA
6.	MSC	56	White	Female	UC	42	MTX
7.	Breast cancer	58	White	Female	CD	56	AZA
8.	Poorly differentiated carcinoma	80	White	Male	CD	73	AZA
9.	Prostate	58	Indian	Male	CD	48	AZA
10.	NMSC	52	White	Male	UC	50	AZA

IBD=inflammatory bowel disease. UC=Crohn's disease. UC=ulcerative colitis. RCC=renal cell carcinoma. NMSC=non-melanoma skin cancer. MSC=melanoma skin cancer. AZA=azathioprine. MTX=methotrexate.

CHAPTER 4

4.1 Discussion

There was an increase in risk of malignancy in IBD patients on IS therapy. Older age at diagnosis significantly increased the risk. Although duration of exposure contributed to a high prevalence of malignancies in this cohort, this was not statistically significant (p=0.641). Eight of the malignancies occurred in patients exposed to immunosuppression for over 2 years. NMSC and RCC were the two most common malignancies and thus should be considered as potential adverse effects in IBD patients on immunosuppression.

Thiopurines (AZA, 6-MP) are the mainstay IS therapies in the management of IBD and its complications (5). Treatment goals of rapid induction (often with high doses) and maintenance of remission often come with potential adverse effects. There is considerable amount of evidence that thiopurines are associated with carcinogenesis (8, 16-19, 36, 59). A number of studies have looked into the risk of diferent cancers in IBD cohorts on IS therapy. One such study done by the CESAME study group have shown 5-fold higher risk of lymphoproliferative disorders (LPD) compared to patients not exposed to these drugs (23). Whether the excess risk was due to IBD itself (21, 60), thiopurines or the combination could not be ascertained. Most patients on IS therapy have failed 5-amanisalicylic acids (5-ASA) and steroids due to severe inflammatory

activity. Perhaps patients with severe inflammation were over-represented and that was a potential confounder.

There was one UC patient in this study who developed follicular gastric lymphoma 10 years after IBD diagnosis. However, this patient was not on immunosuppression. It was postulated that this was perhaps caused by another etiology such as Helicobacter pylori infection (61) as UC does not involve the gastric mucosa. Out of the four cases of NHL in a study by Farrell, 3 cases were of intestinal origin (29). Despite the fact that these patients were all exposed to IS therapy, a type II error and longstanding extensive UC could also explain the 59-fold excess risk (23, 60). Similar to this current study research, the study done by Farrell was limited by a small sample size. Their overall absolute risk of developing NHL in patients on IS therapy was as low as 0.06% per patient-years.

None of the patients exposed to IS therapy developed a lymphoma (whether intestinal or extra-intestinal) in our cohort. This is in contrast to several studies which have shown a significant risk (18, 20, 22, 23, 27, 30). Most of them were prospective observational cohort studies and were highly powered. Nonetheless, there have been studies that have failed to show an association between cancer and immunosuppression in IBD populations. Although most of them were retrospective in design (21, 62-64), there was a meta-analysis (65) and some prospectively designed studies (66, 67). Masunaga and colleagues, in 2007, included 9 moderate to high quality cohort studies in their meta-analysis (65). Only 3 of the 9 studies had control groups. Exposure and duration of

exposure to IS therapy did not significantly increase risk of cancer compared to those not receiving IS therapy. However, this meta-analysis had several drawbacks. Firstly, some studies had different protocols on drug dosages and length of drug exposure (1.0-7.4 years). Secondly, patient adherence could not be verified as this can have a major impact on the occurrence of cancer.

In an observational study in Spain with included 812 patients (52.83% exposed to thiopurines), the risk of cancer in IBD patients exposed to thiopurines compared to those who were not, was not elevated (p=0.013) (64). Most patients (79.5%) were on AZA for a mean duration of 72.16 ±55.7 months. It took an average time of about 5.5 years from initiation of thiopurines to the development of cancer. NMSC, lymphoma and prostate cancer were the most common malignancies in patients exposed to IS therapy. However, there were more cancers in the untreated group (8.1% vs 4%). The reason for this observation is that in the untreated group there was a higher number of CRC (mostly in men with UC) and breast cancer. In fact, none of the patients on thiopurines had these aforementioned malignancies. It is a well-known fact that extensive, longstanding and active UC increases risk of CRC through dysplastic transformation of the inflamed mucosa and immunosuppressive therapy is protective (68).

Although the one breast cancer case in this study was exposed to azathioprine, there is little evidence that breast cancer rates increase with exposure to thiopurines or IBD. In fact, the risk might be similar to that of the general population (64, 69). However, data

on immunosuppression and cervical neoplasia particularly in the setting of HIV (53) and solid organ transplant (54) is adequate. Human papillomavirus subtypes 16 and 18 are potent oncogenic viruses that have an unchecked and unrestricted mutagenic activity in the setting of immunosuppression (55).

There is paucity of data linking IBD and thiopurine therapy to cervical abnormalities. Few studies have looked into the association of IBD (without IS therapy exposure) and cervical cancer but found no statistical significant increase in risk (57, 58, 69). Very few studies have stratified the risk by medication exposure and current evidence is not conclusive. In a recent meta-analysis by Allegretti, the risk of high-grade cervical dysplasia and cervical cancer in IBD patients on IS therapy was increased compared to the general population (OR=1.34, 95% CI: 1.23-1.46). However, all 9 studies in this meta-analysis had different study designs and control group characteristics and did not stratify the risk according to disease subtype (UC vs. CD). Other risk factors such as oral contraceptive use, multiple sexual partners and smoking were not taken into account by some of these studies. Although they managed to capture all relevant studies from multiple databases addressing this association, they failed to further stratify the risk by the drug class, level and duration of immunosuppression.

In this study only 6 out the 104 women had cervical Pap smears and none had more than low-grade dysplasia regardless of medication exposure. It was noticed that Pap smears were not routinely done and HPV status was unknown in those screened for

cervical lesions. A majority of high grade lesions might have been missed. This underscores the need for prospective studies in African IBD population, stratifying for disease subtype, drug class, dosage, duration of IS therapy and whether HPV serology further alters the risk.

There were two cases (22.22%) of biopsy-proven NMSC out of the 99 patients who were exposed to IS therapy. Both were elderly white patients exposed to AZA for over 4 years. They were subsequently changed to MTX and tacrolimus respectively. The use of the aforementioned drug classes has not been significantly linked with NMSC in the IBD population (40). Unfortunately there were no chart records suggesting that these malignancies had regressed after substitution of therapy. None of the black patients who were users and non-users of thiopurines developed skin cancer. It is not known whether black skin tone in South African IBD patient population is protective, therefore a longitudinal prospective study is needed for clarification.

UV radiation and white race are well known risk factors for skin cancer (70). Pathogenesis of skin cancer in AZA treated patients is clearly understood (38). In transplant literature, risk of skin cancer is unequivocal (42). There is a strong body of evidence supporting the association of immunosuppression and NMSC in IBD patients (39, 41, 43, 45). The dose and duration of thiopurine is directly related to development of skin cancer. There was a significant risk with recent and prolonged use of thiopurines in a study by Long published in 2010 with over 50 000 participants (40). This was a well

powered study in terms of sample size and the study population was geographically diverse but they did not have data on race or ethnicity. Detection bias was also a concern because of close contact of IBD patients to health practitioners leading to increased biopsy rates of any suspicious lesion.

Two (22.22%) out of the 99 patients exposed to IS therapy in this study were found to have renal cell carcinoma (RCC). They were male and female and both were over the age of 50 years. However, because of a small sample size and unknown smoking status of the patients, it is difficult to draw any associations. Despite these limitations, there is one prospective observational study which has shown an increased risk of urinary tract cancers in men over the age of 65 years with IBD exposed to immunosuppressants (51). Male gender, advanced age and smoking were independent risk factors. Ten and six cases of kidney and bladder cancer, respectively, were diagnosed with an incidence of 9.6/1000 patient-years. Most kidney cancers were discovered incidentally due to increased imaging studies in these patients for other indications. This could have resulted in over-representation of asymptomatic cancers in patients exposed to thiopurines.

CHAPTER 5

5.1 LIMITATIONS AND STRENGTHS

Perhaps the major limitation of this study was the small sample size. Out of the possibly IBD 250 files in IBD clinic electronic database we managed to find 202 of which 12 were excluded for reasons mentioned above. In the defense of this study, the prevalence of IBD in South Africa is not well known and is expected to be very low. As this clinic is one of the only two referral state clnics for IBD patients in Johannesburg we expected the numbers to be high because of referral bias but it was not the case perhaps reflecting the low prevalence of the IBD in this community. Relying on information from a clinic file is always challenging as crucial data may have been omitted or poorly documented because of the different levels of clinicians seeing the same patients and the fact that we do not have a standard clerking format sheet. The researcher only relied on two government laboratory services to confirm the diagnosis of IBD and to search for cancer. There is a possibility that some patients might have had IS therapy-related cancer diagnosed in private health institutions and there was no direct access to private laboratory data. The retrospective design of the study was also a limitation. There was no control group and we were unable to ascertain the IS therapy dose and adherence to pharmacotherapy as these have major impact on carcinogenesis. It was noted that cervical smear screening was not routinely done as per protocols. This might explain why high grade lesions were not observed.

CHAPTER 6

6.1 CONCLUSION AND RECOMMENDATIONS

This study found a high prevalence of malignancies in IBD patients exposed to immunosuppression compared to those who were not. Older age at diagnosis and duration of treatment were other risk factors. Because of the small power of this study and its retrospective design it is difficult to formulate any recommendations to guide clinicians on cancer risk reduction and steps to follow when cancer does develop. The risk should be weighed against potential benefits. Nevertheless, it is important to adhere to current European Crohn's and Colitis Organisation group guidelines on cancer screening and risk reduction (66):

1. IBD- related lymphoproliferative disorders: (i) check EBV viral load in patients with clinical suspicion, (ii) avoid thiopurines (prefer other immunosuppressants) in EBV-negative young males, (iii) avoid a prolonged combination of anti-TNF therapy and thiopurines beyond 2 years in young men to limit risk of hepatosplenic T-cell lymphoma (HSTCL).
2. Cervical abnormalities: (i) smoking cessation, avoid long term combination oral contraceptive pill, counsel on sexual practices, (ii) obtain Pap smear twice 1st year after diagnosis, if normal then annually, (iii) HPV vaccine should be implemented in all women between 9 and 26 years.

3. Skin cancer: (i) regular dermatological screening and protection again UV radiation for lifelong in all IBD patients in IS therapy, (ii) substituting thiopurines to MTX or calcineurin inhibitors in patients with skin cancer.

APPENDICES

Appendix 1

RESEARCH PROTOCOL

PREVANCE OF MALIGNANCIES IN INFLAMMATORY BOWEL DISEASE PATIENTS ON IMMUNOSUPPRESSANTS

Researcher: Dr L Sobazile

Student number: 1586450

Registered degree: MMED (Internal Medicine)

Supervisors: 1. Dr M. E Seabi MBChB (Medunsa), FCP (SA), MMED (WITS), Cert. Gastro (SA). Specialist Physician at CMJAH Gastroenterology

2. Dr W. F Mudombi MBChB (University of Zimbabwe) MMED (Medicine) (University of Zimbabwe), Cert. Gastro (SA)

Specialist Physician at CMJAH Gastroenterology

INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) are idiopathic chronic inflammatory bowel diseases (IBD) affecting approximately 0.4% of the North American and West European population (1). Immunomodulatory therapy is used both to induce and maintain remission. Immunosuppressive therapy is essential for preventing inflammation-related malignancies but at the expense of interfering with the immune system. Given the prolonged and sometimes lifelong courses of such therapies and the ageing IBD population, the risk of carcinogenesis is increased (1).

The association between chronic active colitis and colorectal cancer (CRC) is well established. There is a 1.5 to 2 fold increased risk of CRC in IBD patients living in North America (2). The dysplasia-carcinoma sequence is the most implicated pathogenetic mechanism in chronic colitis (3). Incidence of CRC appears not to be increased with the wide use of immunomodulatory therapy (4, 5).

Thiopurines (azathioprine, 6-mercaptopurine), methotrexate and TNF-α antagonists are therapies widely used to control intestinal inflammation. An increasing number of patients with CD and some patients with UC are enrolled on these therapies. Biologic agents are not available in our setting and their association with malignancies will not form part of this study.

Immunosuppressants, after a long-term exposure, exert their carcinogenicity by directly altering cellular DNA, activation of oncogenes, impairing immune control of chronic infection by mutagenic viruses and reducing tumor surveillance (6,7). The duration and degree of

immunosuppression among other host factors such as age, gender and prior malignancies are important risk factors for the development of new or recurrent malignancies (1,4,5).

Immunosuppressants increase both the incidence of solid and hematological malignancies (1,4,8,9). Allegretti et al, in a recent meta-analysis showed sufficient evidence to suggest an increased risk of high grade cervical dysplasia/cancer in patients with IBD on immunosuppressants compared to the general population. There is high risk of lymphoma in patients seropositive for EBV (10,11). Risk of developing NHL from IBD itself is negligible (12,15), except for a primary intestinal lymphoma strongly associated with the increased duration of bowel inflammation (14). The CESAME study has shown a 5-fold incident risk of developing lymphoproliferative disorders in patients exposed to thiopurines (15). Several studies have showed an increased risk of acute myeloid leukemia and myelodysplastic syndromes in past users of thiopurines (14,16).

Numerous studies have consistently shown an increased risk of non-melanoma skin cancers [(NMSC) (basal-cell and squamous-cell carcinomas)] in IBD patients on immunosuppressants (6,17,18,19,20). It has been proven that thiopurines increased toxicity of ultraviolet A radiation on epithelial skin cells (6,7). Long et al, in a cohort study in 2012, concluded that immunosuppression increases the risk of melanoma and NMSC among patients with IBD (IRR, 1.29;95% CI, 1.09-1.53 and IRR, 1.46;95% CI, 1.40-1.53 respectively). In recipients of solid organ transplants on immunosuppression the risk of NMSC, SCC in particular, is 65 to 250 fold (24). IBD on its own increases risk of melanoma and thiopurine therapy appears not to alter this

poorly understood risk (17,19). Therefore, the risk of melanoma due to thiopurines remains controversial. Patients on TNFα antagonists have a 1.5 to 2 fold risk of melanoma (20).

However, the association between immunosuppression therapy in IBD patients and malignancies appears to be controversial. A multi-center retrospective study in Japan suggested that thiopurine therapy may not increase the risk of hematological malignancies (22). A large case-control study did not show that that the risk of non-Hodgkin's lymphoma was increased (25). Gomez-Garcia et al also did not find an increased risk of malignancies associated with thiopurine use (23). There is a paucity of data with use of methotrexate and there has not been conclusive evidence that it increases risk of cancer (26).

Therefore, in the face of existing controversies, it is of clinical benefit to quantitatively measure the risk of different malignancies in IBD patients who use immunosuppressive therapy in our local setting. Data at our disposal comes from studies done mostly in Europe and North America but not in Africa. If this becomes a positive study, risk reduction strategies such as routine skin surveillance and use of sunscreen for prevention of skin cancer, regular cervical and anal PAP smear, routine EBV serological testing and HPV vaccination will need to be implemented and firmly adhered to. It is also fundamental to develop risk-benefit protocols stratified for age, sex and IBD phenotype in planning long term immunosuppressive therapy.

OBJECTIVES

1. To determine the prevalence of malignancies in IBD patients on immunosuppressants attending CMJAH gastroenterology clinic.
2. To determine if the duration of treatment, age at diagnosis, gender and race are related to the development of malignancies.
3. To compare the prevalence of malignancies based on the disease entity: ulcerative colitis vs Crohn's disease (both being treated by immunomodulatory therapy).
4. To compare the prevalence of malignancies between IBD patients on and not on immunomodulatory therapy.

METHODOLOGY

Study Design: Retrospective record review

Data materials:

All files in our IBD Registry at CMJAH Gastroenterology Department will be reviewed. We keep all IBD patient files at our record room in Area 554. Our filing system is in alphabetical order to make file retrieval more convenient. All IBD patients' clinic files will be included in this study even if they are not on immunosuppressants (azathioprine, 6-mercaptopurine, methotrexate, tacrolimus, and cyclosporine). For each IBD patient I will then go through National Health Laboratory Service and Disa*Lab to access histopathology results to confirm the diagnosis of IBD and cancer if present.

Inclusion criteria:

i. Age 18 years or older.
ii. All racial groups and gender.
iii. Endoscopy- and/or histology diagnosed IBD.
iv. Patients on any of the following drugs: thiopurines (Azathioprine and 6-MP), methotrexate, calcineurin inhibitors (tacrolimus and cyclosporine).
v. At least 6 months on immunosuppressive therapy.
vi. IBD diagnosis made between year 1990 and January 2017.

Exclusion criteria:

i. HIV seropositive patients.
ii. Patients with pre-existing malignancies prior treatment with immunomodulatory therapy.
iii. Patients with indeterminate colitis

DATA ANALYSIS

Descriptive statistics will be used to analyze patients on or not on immunosuppressants. Conditional logistic regression will be used to determine associations between medications and cancer. Continuous variables will be reported as median with inter-quartile ranges. Categorical variables will be reported as percentages. Kaplan Meier Survival plots will be used to determine time to development of malignancies. To calculate the p value Kruskal Wallis test will be used. A p value of < 0.05 will be regarded as statistically significant and the null hypothesis will disregarded.

ETHICS

Copies of this protocol, ethics application forms and relevant attachments will be submitted to the Human Research Ethics Committee for clearance.

TIMING

The study will commence as soon as it is approved by the Wits Ethics Committee. The study duration will be for a period of 1 year

FUNDING

No funding will be required for any costs emanating from this study.

LIMITATIONS

- There is a possibility that some files might be lost or kept else.
- Poorly recorded data on patient files.
- Excluding HIV seropositive patients may reduce sample size.
- Cervical and anal PAP smears not routinely done.
- EBV and HPV serology not routinely done.

APPENDIX 2

DATA COLLECTION SHEET

Demographics

Age (years) ☐

Gender Male ☐ Female ☐

Race Black ☐ White ☐ Other ☐

IBD Phenotype

Ulcerative colitis ☐ Crohn's disease ☐

Age of diagnosis ☐

Duration of disease ☐

Treatment: Yes ☐ No ☐

Treatment received: Thiopurine (Azathioprine, 6-MP) ☐ Methotrexate ☐

Calcinuerin inhibitors (Tacrolimus, cyclosporine) ☐

Duration of treatment: Months ☐ Years ☐

Malignancies

Any cancer diagnosed before IBD immunosuppressive therapy? Yes ☐ No ☐

Any cancer diagnosed during the course of above treatment? Yes ☐ No ☐

Type of cancer

Mode of diagnosis:

Is above mode gold standard? Yes ☐ No ☐

APPENDIX 3: ETHICS CLEARANCE CERTIFICATE

R14/49 Dr Loyiso Sobazile

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M160917

NAME: (Principal Investigator)	Dr Loyiso Sobazile
DEPARTMENT:	Internal Medicine Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Prevalence of Malignancies in Inflammatory Bowel Disease Patients on Immunosuppressants
DATE CONSIDERED:	30/09/2016
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr M.E Seabi and Dr W.F Mudombi
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	25/01/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in September and will therefore be due in the month of

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